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Ottawa Hull K1A 0C9

(21) (A1) 2,109,239
(22) 1993/10/26
(43) 1994/04/28

(51) INTL.CL.[5] G01N-033/569; G01N-033/577

(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

5,091,048

(54) Method For the Simultaneous Determination of Antigens and Antibodies

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(30) (DE) P 42 36 189.3 1992/10/27

(57) 33 Claims

ABSTRACT

For the simultaneous determination of an antigen of a pathogen and at least one antibody against the same pathogen based on the principle of a heterogeneous immunoassay, the sample is incubated with receptors R1 to R4 whereby R1 and R3 are capable of specific binding to the antigen to be determined, R2 and R4 are capable of specific binding to the antibody to be determined, R1 and R2 mediate binding to the solid phase and R3 and R4 carry the same label and, after separating the solid from the liquid phase, the label is determined in one of the two phases.

Method for the simultaneous determination of antigens and antibodies

The invention concerns a method for the simultaneous determination of at least one antigen of a pathogen and at least one antibody against the same pathogen based on the principle of a heterogeneous immunoassay by incubating the sample with the receptors R1 to R4, in which R1 and R3 are capable of specific binding to the antigen to be determined, R2 and R4 are capable of specific binding to the antibody to be determined, R1 and R2 mediate binding to the solid phase, R3 and R4 carry the same label, separating the solid phase from the liquid phase and determining the label in one of the two phases.

Numerous important clinical parameters are determined with immunological detection methods. There is a wide selection of homogeneous and heterogeneous methods for immunoassays. Often a heterogeneous method based on the sandwich principle or a variant derived from this is used. The sample solution is usually incubated with a receptor which is capable of binding to the substance to be determined and carries a label and with a receptor capable of specific binding to the substance to be determined which is bound to the solid phase or mediates binding to the solid phase. In this process bound complexes of the substance to be determined and labelled receptor are formed on the solid phase via the receptor mediating binding to the solid phase. After separating the liquid from the solid phase, the label can be detected in one of the two phases.

In order to detect an infection by a pathogen, either antibodies which are specifically directed towards this pathogen or certain antigens of this pathogen are usually detected. Separate determinations of the antibodies and antigens are carried out in order to detect an infection as reliably as possible. These tests have a lower detection limit. If measured signals are obtained in the test which are below this detection limit then this sample is declared to be negative. If the measured signals are above the detection limit then the sample is declared to be positive. When the samples are ambiguous it is often necessary to repeat the measurement. Despite this, some samples which only contain small amounts of antigen or antibody are falsely classified as negative by this limitation of the detectability of the individual substances to be determined. This is a particular problem for the early detection of infection parameters.

The object of the present invention was to develop an immunoassay for the determination of antigens of a pathogen and antibodies against this pathogen which enables an improved differentiation between negative and positive samples.

This object is achieved by a method for the simultaneous determination of at least one antigen of a pathogen and at least one antibody against the same pathogen based on the principle of a heterogeneous immunoassay by incubating the sample with at least one receptor R1 which is capable of specific binding to the antigen to be determined that is not identical to the antigen of the pathogen towards which the antibody to be determined is directed and is bound to or can be bound to a solid phase, with at least one receptor R2 which is capable of

specific binding to the antibody to be determined and is bound to or can be bound to a solid phase, with at least one receptor R3 which is capable of specific binding to the antigen to be determined that is not identical to the antigen of the pathogen towards which the antibody to be determined is directed and carries a label and with at least one receptor R4 which is capable of specific binding to the antibody to be determined and carries the same label as the receptor R3, separating the solid from the liquid phase and determining the label in one of the two phases.

A method for the determination of several substances i.e. several antibodies or antigens or antigens of a pathogen in combination with antibodies against another pathogen in an immunoassay is described in EP-A 0 379 216. Several receptors R1 are used which bind specifically to the substances to be determined. The receptors R1 mediate binding to the solid phase. In addition various receptors R2 can be used which are capable of binding to various substances to be determined. The receptors R2 either carry different labels or the same label. If different labels are used, the method can be carried out in a single phase, i.e. the receptors R2 are added simultaneously. If the same label is used, the method must be carried out in two phases i.e. the various receptors R2 are added successively and the individual labels are determined successively in this way. A simultaneous determination of antigens of a particular pathogen and antibodies which are directed towards this particular pathogen and which enables a good differentiation between negative and positive samples is not disclosed in EP-A-0 379 216.

The simultaneous determination of antigens of a pathogen and antibodies which are directed towards this particular pathogen is made possible since, according to the present invention, those receptors are used as receptors R1 and R3 which specifically bind the antigen to be determined but are not directed towards an antigen against which the antibody to be determined is directed. If receptors were used which also specifically bind the antigen that is bound by the antibody to be determined then a mutual neutralization of antigens and antibodies would occur.

Since the method according to the present invention enables the simultaneous detection of antigens of a pathogen and antibodies that are directed towards this pathogen pathogen in samples, there is a summation of the measured signals caused by the antigens and antibodies. Samples with a low content of antigens and antibodies which yield borderline signals when the antigens or antibodies are determined separately, i.e. signals which are near to the detection limit and which may be falsely classified as negative or require a repeated determination, can be classified as positive using the method according to the present invention. This therefore obviates the time-consuming repeated determination. In addition after an infection has occurred it is possible to detect patients earlier as being infected and thus to start treatment earlier. This was surprising since it was assumed that at the beginning of an infection only antigens can be detected.

When selecting receptors R1 to R4 it is important, in case the method is intended for an early detection of infections, to use those receptors which are directed towards early antigens i.e. antigens which occur very

soon after infection or are directed towards antibodies which occur very soon after infection in high titres in the samples. If it is only intended to enable an improved differentiation between negative and positive samples using the method according to the present invention and it does not serve specifically for the early detection of infectious diseases, but instead for example the monitoring of an infectious disease, it is also possible to use receptors R1 to R4 which are directed towards antigens or antibodies which do not occur until later in the course of an infection. The selection of the receptors therefore depends on which stage of the infectious disease is intended to be determined. The method according to the present invention is particularly suitable for the early detection of infectious diseases.

The method according to the present invention is carried out according to the principle of a heterogeneous immunoassay. A solid phase is used on the surface of which the receptors R1 and R2 are directly bound or can be bound.

Known materials such as plastic, glass, paper carriers, ceramics, latex and magnetic particles can be used for the solid phase. The solid phase can for example be present in the form of reaction tubes, reagent carrier strips or spheres. In a preferred embodiment the solid phase is present in the form of a reaction tube the inner surface of whose walls is or can be at least partially coated with the receptors R1 and R2. Known materials are suitable as the material for the reaction vessel. Polystyrene, copolymers of polystyrene, polycarbonates, polyacrylates and polymethacrylates are preferred.

The coating of the solid phase with receptors R1 and R2 is carried out either directly or indirectly. Processes for the direct binding of receptors to a solid phase are known to a person skilled in the art. Adsorptive binding is preferred.

Indirect binding is understood to mean that receptors R1 and R2 are bound to the solid phase only shortly before or during the immunological reaction. In this case a partner P1 of a specific binding pair P1/P2 is bound to the solid phase. The other partner P2 of the specific binding pair is coupled in each case to the receptors R1 and R2.

The coating of the solid phase with the specific binding partner P1 can either be carried out directly, via a carrier material or a spacer. Binding to a soluble protein with a molecular weight over 500000 which is then adsorbed to the inner surface of the reaction vessel is for example suitable. Binding via a spacer which can be covalently or adsorptively bound to the surface of the reaction vessel via a functional group is also suitable. Processes and agents for this are known to a person skilled in the art. In a preferred embodiment a carrier material is used as the solid phase which has been manufactured according to the process described in DE-A-3640412.8. In a further preferred embodiment the solid phase is a reagent carrier that has been obtained by activating a fibre fleece made of a cellulose/synthetic fibre mixture by treatment with periodate and coated with the specific binding partner P1 which was previously treated with acid. A process for the manufacture of such reagent carriers is described in DE-A-3543749.

Antigen/antibody, hapten/antibody, lectin/carbohydrate, biotin/anti-biotin antibody, biotin/avidin, biotin/streptavidin are for example suitable as specific binding pairs P1/P2. Partners capable of binding to biotin, in particular streptavidin or avidin are preferably immobilized on the solid phase.

The quantity ratio of the receptors R1 and R2 bound directly or indirectly to the solid phase can be varied over a wide range. The preferred quantity ratio of the receptors R1 to R2 is between 10:1 and 1:10. The optimal quantity ratio R1:R2 for each special test can be easily determined by binding different amounts of R1 and R2 to the solid phase. If a specific binding pair P1/P2 is used to bind the receptors R1 and R2 to the solid phase, the quantity ratio of the bound receptors R1 and R2 corresponds approximately to the mixing ratio of the receptors R1 and R2 in the solution that is used for the coating since the binding affinities of both receptors are approximately equal. If a direct binding of the receptors R1 and R2 is carried out, the quantity ratio of the directly bound receptors R1 and R2 can differ from the mixing ratio of receptors R1 : R2 in the solution that is used for the coating since the receptors often have different affinities to the solid phase. If this is desired, the exact quantity ratio of the receptors R1 and R2 bound to the solid phase can be subsequently determined according to methods known to a person skilled in the art, for example by determining the binding capacity for the antigens and antibodies to be determined. The quantity ratio R3:R4 is also selected in accordance with the quantity ratio selected for the receptors R1:R2. The quantity ratio of the receptors R3:R4 is also between 10:1 to 1:10. The quantity ratio R3:R4 can in this case be the same or similar to the

quantity ratio R1:R2, deviations can, however, also occur. The optimal quantity ratio R3:R4 can easily be determined for each individual test by mixing different amounts of R3 and R4.

Receptors are used as receptor R1 which have binding sites for the antigen to be determined. The antigen to be determined must be different from the antigen which is recognized and bound by the antibody to be determined. The receptor R1 must specifically recognize a different antigenic determinant or a different epitope of the pathogen than is recognized by the antibody to be determined. It is necessary that receptor R1 and the antibody to be determined do not strongly cross-react with the same binding site. The antigenic determinants are known for known pathogens such as for example hepatitis viruses or HIV. For example in order to detect a hepatitis B virus infection, a receptor against the hepatitis B surface antigen (HBsAg) can be used as receptor R1 and simultaneously anti-hepatitis B core antibodies (<HBc>Ab) are detected as the antibodies to be determined. In order to detect a HIV infection, a receptor R1 can for example be used which binds the p24 protein while anti-gp32, gp41 or gp120 antibodies are determined. If the antigenic determinants are not known for a pathogen then suitable receptors R1 that do not compete with the antibody to be determined for the same binding site can be selected by a person skilled in the art based on known preliminary experiments for determining cross-reactivity.

Mixtures of different receptors can also be used as receptors R1 which are capable of specific binding to different antigens of the pathogen. In a test for HIV infection, receptors for the antigenic determinants p24

and gp32 and/or gp41 can for example be used. In this case an anti-gp120 antibody is for example selected as the antibody to be determined. In the case of a test for a hepatitis B virus infection, a mixture of two receptors for HBsAg and HBcAg can for example be used as receptor R1 while the antibody to be determined is directed towards another epitope of HBcAg or against HBeAG.

Complete antibodies of all subclasses which are capable of binding to the antigen to be determined can be used as receptors R1. Of course their fragments such as Fab, Fab' or $F(ab')_2$ fragments can also be used instead of the complete antibodies. The antibodies can be polyclonal or monoclonal. Since there should be no cross-reactivity with the antibodies to be determined it is advantageous to use highly purified specific polyclonal antibodies. The use of monoclonal antibodies is most suitable.

In a preferred embodiment of the method according to the present invention a partner P2 of the specific binding pair P1/P2 is coupled to the receptor R1. Immobilization on the solid phase is effected via the partner P2 which binds to the partner P1. A hapten such as FITC, p-nitrophenol, saponin, digoxin and particularly preferably biotin is preferably used as the partner P1. Methods for coupling partner P2 to the receptor R2 are known to a person skilled in the art.

The antigens are utilized as receptor R2 to which the antibody or antibodies to be determined specifically bind. Complete or purified parts of pathogens can be used as the antigen such as antigens, protozoal antigens or viral antigens. The antigens can be comprised of

native material, recombinant material or of chemically synthesized or modified proteins or carbohydrates. Thus for example in a test for HIV antibodies different antigenic determinants such as p24, gp41, gp32 or gp120 are used as receptor R2. In a test for hepatitis diseases, the viral antigens HBcAg, HBsAg, HBeAg, HAV etc can be used. As described above the selection of the receptor R2 depends on the receptor R1 used.

As described for receptor R1, a mixture of different receptors can also likewise be used for receptor R2 which are capable of specific binding to different antibodies which are directed towards different antigenic determinants of a pathogen. For example a mixture of the viral antigens gp32, gp41 and gp120 can be used in a test for a HIV infection. In this case receptor R1 would for example be directed towards p24.

Receptor R2 is either directly or indirectly bound to the solid phase. In the preferred embodiment of the method according to the present invention a partner P2 of the specific binding pair P1/P2 is coupled to the receptor R2. In a particular test the same substances should be coupled as partners P2 to the receptor R2 as to the receptor R1. The selection of the partner P2 from various substances and the coupling is carried out in an analogous manner to that described for receptor R1.

The receptors which are used for receptor R3 are analogous to receptor R1 those which have binding sites for the antigen to be determined but are not directed towards the antigen which is specifically bound by the antibody to be determined. The same antibodies can be used as receptor R3 which are also used for receptor R1. In a particular test the antibodies used for receptor R3

can be identical to the antibodies used for receptor R1 or different from this depending on the determination to be carried out i.e. the infection to be detected. For example a complete antibody can be used as receptor R1 and an antibody fragment as receptor R3 which both specifically bind to the same antigen. Receptors R1 and R3 must specifically bind the same antigen. However, in doing so they can bind to different epitopes of the same antigen. It is preferred that receptors R1 and R3 do not compete for the same epitope of the same antigen. This condition can particularly easily be fulfilled when monoclonal antibodies are used as R1 and R3. As for receptor R1, a mixture of different receptors can also be used for receptor R3.

Receptor R3 in addition carries a label. There are many possibilities known for labelling which are suitable in the method according to the present invention. For example radioactive isotopes such as ^{125}I, ^{121}I, ^{51}Cr, ^{35}S and ^{3}H, enzymes such as peroxidase, ß-galactosidase or alkaline phosphatase, fluorescent or chemiluminescent substances or substances which can be detected in another manner can be used. Apart from a direct label, receptor R3 can also carry an indirect label by binding a labelled component capable of binding receptor R3 to this receptor during the course of the test procedure. A labelled antibody which binds the Fc part of receptor R3 can for example be used. Coupling via a hapten antibody binding is preferred. In this case a hapten such as digoxin is coupled to the receptor. A labelled anti-hapten antibody such as an anti-digoxin antibody-peroxidase conjugate binds to the hapten.

A receptor is used as receptor R4 which is capable of specific binding to the antibody to be determined and

which carries the same label as receptor R3. The same detectable substances as defined for R3 are to be used as the label. The same antigens which are also used for receptor R2 can be used as receptor R4. A mixture of different receptors can be used for receptor R4 as in the case of receptor R2.

In a further variant of the method an antibody or a fragment thereof which specifically recognizes the antibody to be determined can be used for the receptor. There are two possibilities for this variant of the method. The first possibility is that an antibody is used as receptor R4 which only specifically recognizes the antibody to be determined and does not bind to receptors R1 and R3. This can for example be achieved by using an antibody as receptor R4 which specifically recognizes human antibodies and using antibodies solely as receptors R1 and R3 which are not of human origin, such as mouse antibodies.

In the second variant of the method an antibody is used as receptor R4 which specifically recognizes the antibody to be determined as well as receptor R3 but does not recognize receptor R1. An antibody, preferably a Fab or Fab' fragment, can for example be used as receptor R4 which specifically binds the Fc part of an antibody. In this case an antibody fragment is used as receptor R1 which no longer contains an Fc part. A complete antibody must in this case be used as receptor R3. Receptor R3 thus does not carry a label which is directly coupled to it but instead receives the label via binding to receptor R4 which carries a label. Receptor R3 thus carries an indirect label.

In order that receptor R3 does not become cross-linked during the incubation by binding of receptor R4, a monovalent antibody fragment, for example a Fab or Fab' fragment, is preferably used as receptor R4. The sample is preferably firstly incubated with the receptors R1, R2 and R3. After the complexes bound to the solid phase have been formed, it is incubated with receptor R4 after prior removal of excess receptors and subsequently the label is determined.

The sample solution can be incubated simultaneously with all receptors. Simultaneous incubation is preferred when R3 and R4 are directly labelled and receptors R1 and R2 are directly bound to the solid phase. If the receptors R1 and R2 are indirectly bound to the solid phase, these two receptors can firstly be preincubated with the solid phase. The sample is subsequently incubated together with receptors R3 and R4. Further variants of the method are possible and known to a person skilled in the art. The simple procedure enables the determination to be carried out on automated analysers. The method is also suitable for a rapid diagnosis. The simultaneous detection of antigens and antibodies of an infection parameter enables blood samples which contain this risk parameter to be immediately eliminated without the necessity for a more exact diagnosis. On the other hand in examinations a rapid test can be used to check beforehand whether risk factors are present and if this is the case then this can be followed by a more exact diagnosis for example by a DNA test. Thus according to the present invention it is possible to very rapidly determine whether particular indications for a disease are present.

Using the method according to the present invention, it is possible to simultaneously detect at least one antigen of a pathogen and at least one antibody which is capable of binding to the same pathogen. In this connection all pathogens come into consideration as pathogens or infection parameters in which an antigen and antibody titre can be detected in blood, especially viruses such as hepatitis viruses, HIV, CMV, EBV and other human viruses, prokaryotes as well as eukaryotic pathogens and protozoa.

The invention also concerns a reagent for the determination of an antigen of a pathogen and at least one antibody against the same pathogen which contains a solid phase on the surface of which the receptors R1 and R2 are bound or can be bound by interaction of the specific binding pair P1/P2, in each case at least one receptor R1, R2, R3 and R4 as defined above and, if desired, further common auxiliary agents for carrying out a heterogeneous immunoassay such as buffers, detergents, substances for eliminating interferences, stabilizers as well as, if desired, agents for determining the label such as enzyme-substrates like ABTS®.

The invention is elucidated by the following examples.

Example 1

HIV-Ag/anti-HIV test

HIV antigens (p24 antigen) and anti-HIV antibodies (anti-gp32, anti-gp41 and anti-gp120) are determined in a pseudo 2-step immunoassay.

Reagent 1:

- 40 mmol/l phosphate buffer, pH 7.0
- 1 % by weight Nonidet® P40

Reagent 2:

- 40 mmol/l phosphate buffer, pH 7.0
- 0.9 % by weight NaCl
- 0.2 % by weight bovine serum albumin
- 100 ng/ml each of one or several biotinylated HIV antigens (gp32, gp41 or gp120)
  50 ng/ml each of one or several digoxin-labelled HIV antigens (gp32, gp41 or gp120)
- 150 ng/ml biotinylated anti-HIV p24 antibody
- 75 ng/ml digoxin-labelled anti-HIV p24 antibody
- 150 mU/ml anti-digoxin antibody-peroxidase conjugate

The following antigens were used in this process:

- chemically synthesized peptides
- HIV I gp120 (V3 loop) (EP-A 0 311 219)
- HIV II gp 41 (Gnann, J.W. et al., Science 237, 1987, 1346)
- HIV III gp32 (EP-A 0 379 216)

Purified human polyclonal anti-p24 antibodies (Innogenetics Co.) were used as the antibodies.

The antigens and antibodies were labelled with biotin or digoxin as described by Leary et al., PNAS, 80 (1983), 4045.

The test was carried out in a streptavidin thermo-BSA polystyrene tube as described in EP-A 0 379 216.

100 µl human serum or human plasma was incubated with 200 µl reagent 1 for 60 minutes at 37°C in the streptavidin thermo BSA polystyrene tube. Subsequently 700 µl reagent 2 was added and incubated for 120 minutes at 37°C. Afterwards it was washed three times with tap water and 1 ml ABTS® substrate solution was added for the test reaction. After a 60 minute incubation at 37°C the absorbance was measured photometrically at 422 nm.

The anti-HIV test was carried out using the individual HIV antigens and a combination of all HIV antigens. In doing so it turned out that the test procedure with streptavidin thermo-BSA conjugate polystyrene tubes is suitable for the simultaneous determination of several individual antibodies or an antibody population and antigens regardless of whether the antibodies are directed towards the same virus or several viruses or their antigens and of whether the detected antigens belong to one or several viral strains (Table 1).

Table 1

HIV antigens and antibodies	Negative control	Human serum samples				
		HIV I Ab+Ag	HIV I Ab+Ag	HIV I Ab+Ag	HIV I Ab+Ag	HIV II Ab+Ag
		(absorbances in mA)				
gp 120 (pep)	52	2161	5611	3861	94	63
gp 41 (Pep)	48	2555	5735	2872	89	72
gp 32 (Pep)	44	54	247	64	41	1389
<p24> Ab	56	367	427	76	512	1597
peptides+ <p24>Ab	62	4123	6895	5678	643	2763

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. Method for a simultaneous determination of at least one antigen of a pathogen and at least one antibody against said pathogen based on a principle of a heterogeneous immunoassay, wherein a sample is incubated with:

(I) at least one receptor R1 which is capable of specific binding to a first antigen to be determined, wherein said antigen is not identical to a second antigen of the pathogen and is bound to or can be bound to a solid phase,

(II) at least one receptor R2 which is capable of specific binding to the antibody to be determined and which is bound to or can be bound to a solid phase,

(III) at least one receptor R3 which is capable of specific binding to said first antigen to be determined, wherein said first antigen is not identical to said second antigen of the pathogen towards which the antibody to be determined is directed and which carries a label,

(IV) at least one receptor R4 which is capable of specific binding to the antibody to be determined and carries the same label as receptor R3,

separating said solid phase from a liquid phase and determining the label in one of said phases.

2. Method as claimed in claim 1, wherein each of the receptors R1 and R3 is a mixture of different receptors which are capable of specific binding to different antigens of a pathogen to be determined.

3. Method as claimed in claim 1 or 2, wherein the receptors R1 and R3 are monoclonal or polyclonal antibodies.

4. Method as claimed in claim 3, wherein the receptors R1 and R3 are monoclonal or polyclonal anti-HIV p24 antibodies.

5. Method as claimed in claim 1, 2 or 4, wherein each of the receptors R2 and R4 is a mixture of different receptors which are capable of specific binding to antibodies to be determined.

6. Method as claimed in claim 3, wherein each of the receptors R2 and R4 is a mixture of different receptors which are capable of specific binding to antibodies to be determined.

7. Method as claimed in claim 1, 2, 4 or 6, wherein the receptors R2 and R4 are complete or purified parts of pathogens such as bacterial antigens, protozoal antigens or viral antigens.

8. Method as claimed in claim 3, wherein the receptors R2 and R4 are complete or purified parts of pathogens such as bacterial antigens, protozoal antigens or viral antigens.

9. Method as claimed in claim 5, wherein the receptors R2 and R4 are complete or purified parts of pathogens such as bacterial antigens, protozoal antigens or viral antigens.

10. Method as claimed in claim 7, wherein the viral antigens are synthesized peptides.

11. Method as claimed in claim 8, wherein the viral antigens are synthesized peptides.

12. Method as claimed in claim 9, wherein the viral antigens are synthesized peptides.

13. Method as claimed in claim 10, 11 or 12, wherein the synthesized peptides are the HIV I peptides gp120, gp41 and gp32.

14. Method as claimed in claim 1, 2 or 4, wherein the receptor R4 is an antibody which specifically binds the antibody or antibodies to be determined.

15. Method as claimed in claim 3, wherein the receptor R4 is an antibody which specifically binds the antibody or antibodies to be determined.

16. Method as claimed in claim 1, 2, 4, 6, 8, 9, 10, 11, 12 or 15, wherein a partner P2 of a specific binding pair P1/P2 is coupled to the receptors R1 and R2, the partner P1 of the specific binding pair is bound to the solid phase and the receptors R1 and R2 are bound to the solid phase via the specific binding of P1 to P2.

17. Method as claimed in claim 3, wherein a partner P2 of a specific binding pair P1/P2 is coupled to the receptors R1 and R2, the partner P1 of the specific binding pair is bound to the solid phase and the receptors R1 and R2 are bound to the solid phase via the specific binding of P1 to P2.

18. Method as claimed in claim 5, wherein a partner P2 of a specific binding pair P1/P2 is coupled to the receptors R1 and R2, the partner P1 of the specific binding pair is bound to the solid phase and the receptors R1 and R2 are bound to the solid phase via the specific binding of P1 to P2.

19. Method as claimed in claim 7, wherein a partner P2 of a specific binding pair P1/P2 is coupled to the receptors R1 and R2, the partner P1 of the specific binding pair is bound to the solid phase and the receptors R1 and R2 are bound to the solid phase via the specific binding of P1 to P2.

20. Method as claimed in claim 13, wherein a partner P2 of a specific binding pair P1/P2 is coupled to the receptors R1 and R2, the partner P1 of the specific binding pair is bound to the solid phase and the receptors R1 and R2 are bound to the solid phase via the specific binding of P1 to P2.

21. Method as claimed in claim 14, wherein a partner P2 of a specific binding pair P1/P2 is coupled to the receptors R1 and R2, the partner P1 of the specific binding pair is bound to the solid phase and the receptors R1 and R2 are bound to the solid phase via the specific binding of P1 to P2.

22. Method as claimed in claim 1, 2, 4, 6, 8, 9, 10, 11, 12, 15, 17, 18, 19, 20 or 21, wherein the receptors R3 and R4 carry an indirect label.

23. Method as claimed in claim 3, wherein the receptors R3 and R4 carry an indirect label.

24. Method as claimed in claim 5, wherein the receptors R3 and R4 carry an indirect label.

25. Method as claimed in claim 7, wherein the receptors R3 and R4 carry an indirect label.

26. Method as claimed in claim 13, wherein the receptors R3 and R4 carry an indirect label.

27. Method as claimed in claim 14, wherein the receptors R3 and R4 carry an indirect label.

28. Method as claimed in claim 16, wherein the receptors R3 and R4 carry an indirect label.

29. Method as claimed in claim 22, wherein the indirect label is generated via a hapten-antibody binding in which the hapten is coupled to receptors R3 and R4 and the hapten-specific antibody carries a label.

30. Method as claimed in claim 23, 24, 25, 26, 27 or 28, wherein the indirect label is generated via a hapten-antibody binding in which the hapten is coupled to receptors R3 and R4 and the hapten-specific antibody carries a label.

31. Method as claimed in claim 29, wherein digoxin is used as the hapten and an anti-digoxin antibody peroxidase conjugate is used as the labelled antibody.

32. Method as claimed in claim 30, wherein digoxin is used as the hapten and an anti-digoxin antibody peroxidase conjugate is used as the labelled antibody.

33. Reagent for a determination of at least one antigen of a pathogen and at least one antibody against said pathogen comprising:

(I) a solid phase to which receptors R1 and R2 are bound or can be bound,

(II) at least one receptor R1 which is capable of specific binding to a first antigen to be determined, wherein said first antigen is not identical to a second antigen of the pathogen and is bound to or can be bound to the solid phase,

(III) at least one receptor R2 which is capable of specific binding to the antibody to be determined and is bound to or can be bound to the solid phase,

(IV) at least one receptor R3 which is capable of specific binding to said first antigen to be determined, wherein said first antigen is not identical to said second antigen of the pathogen towards which the antibody to be determined is directed and which carries a label,

(V) at least one receptor R4 which is capable of specific binding to the antibody to be determined and which carries the same label as receptor R3.

SUBSTITUTE

REMPLACEMENT

SECTION is not Present

Cette Section est Absente